Assignment 4

Properties of the Hodgkin-Huxley equations

Submission: Moodle (single compressed file including results/explanations and Matlab files)

This assignment aims to investigate some of the properties of the celebrated Hodgkin-Huxley equations. Experimentally in a space-clamped axon of finite length the regenerative electrical activity in response to some supra-threshold stimulus is called a membrane action potential as the whole axonal membrane simultaneously participates in the events underlying the action potential. Without the space clamp the action potential would propagate with finite velocity away from the site of supra threshold stimulus application and would thus constitute a propagating action potential. This latter event is the one that would occur physiologically. Many of the properties of the propagating action potential are similar, if not identical, to the membrane action potential. The following four features of the Hodgkin-Huxley equations, all of which are observed physiologically, will be simulated in this assignment.

- Threshold

- Refractoriness - absolute and relative

- Repetitive activity

- Temperature dependence

Table 1 shows the parameters and values first articulated by Hodgkin and Huxley in 1952 for the squid giant axon together with the corresponding MATLAB variables to be used. Load the default equation and stimulus parameters by typing the following command in MATLAB.

```
>> hhconst;
```

parameter	MATLAB variable	value	units
$\overline{G}_{Na}$	g_na_max	120	$\mathrm{mS\,cm^{-2}}$
$\overline{G}_{K}$	g_k_max	36	$\mathrm{mS\,cm^{-2}}$
G_L	g_l	0.3	$\mathrm{mS\,cm^{-2}}$
C_{Na}^{o}	co_na	491	$\mathrm{mmol\,L^{-1}}$
C_{K}^{o}	co_k	20.11	$\mathrm{mmol\,L^{-1}}$
C_{Na}^{i}	ci_na	50	$\mathrm{mmol\,L^{-1}}$
C_{K}^{i}	ci_k	400	$\mathrm{mmol\,L^{-1}}$
E_L	e_l	-49	mV
$V_{m,r}$	e_vr	-59.8	mV
C_m	CM	1.0	$\mathrm{\mu F\,cm^{-2}}$
T	tempc	6.3	^{o}C

Table 1 - Original parameters of the Hodgkin-Huxley equations for the giant squid axon.

1. Threshold

An essential property of the action potential is the notion of threshold. The range of stimulating current intensities between sub-threshold behaviour (i.e no action potential generated) and supra threshold behaviour (i.e an action potential is generated) is extremely narrow. The Hodgkin-Huxley equations exhibit similar threshold-like behaviour in response to electrical stimulation.

Early experiments on single axons of the crab Carcinus maenas revealed that relative changes in the stimulating current strength of less than 1% were able to span the entire region between sub- and supra- threshold responses.

Begin by applying a short duration sub-threshold and supra-threshold current pulse to the Hodgkin Huxley equations using the default parameters that you have already loaded.

```
>> amp1 = 6;
>> width1 = 1;
>> hhmplot(0,50,0);
>> amp1 = 7;
>> hhmplot(0,50,1);
```

Visualization of this stimulus in general is shown in Figure 1 and the description of these parameters are given in Table 2.

MATLAB variable	default value	units
delay1	0	ms
width1	40	ms
amp1	5	$\mu A\,cm^{-2}$
delay2	0	ms
width2	0	ms
amp2	0	$\mu A\,cm^{-2}$
vclamp	0	mV

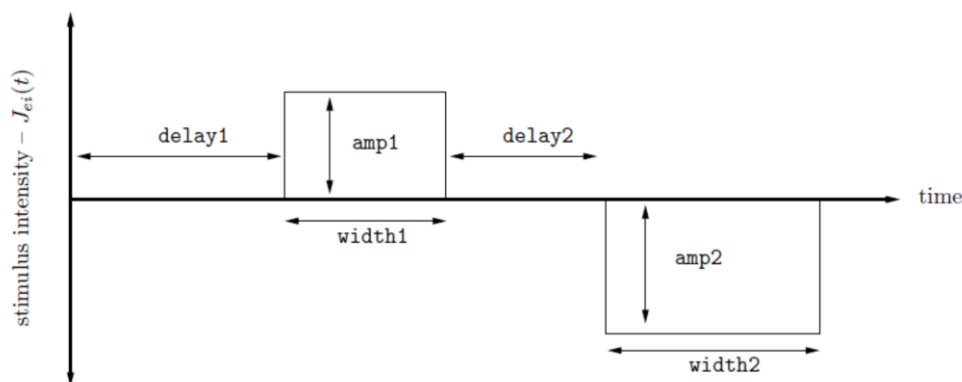*Table 2 - MATLAB variables determining stimulus current.*

Figure 1 - Definitions of stimulus current parameters of Table 2.

NOTE

- All simulation variables can be listed using `whos`.

- The current pulse(s) defined by amp1, amp2 etc. correspond to the external current injection $k_e(t)$, $J_e(t)$ corresponding to lecture notes when `amp1>0`. `amp2>0` will be depolarising.

- The general usage of the command `hhmplot` is `hhmplot(to,tf ,hold)` where t_o and t_f are the times in milliseconds of the beginning and end of the integration interval. `hold = 1` if the output of `hhmplot` is to be added to an existing plot and `hold = 1` if the output of `hhmplot` is to be a new plot.

Question 1

Continue to bisect the amplitude interval for sub-threshold and supra-threshold stimulating currents and obtain an estimate of the threshold stimulating current amplitude to two decimal places.

The function `hhsplot` provides an alternative way of plotting the results of numerical integrations of the Hodgkin-Huxley equations and has as output arguments.

$$\int_{t_o}^{t_f} J_{Na}(t)\ dt, \quad \int_{t_o}^{t_f} J_K(t)\ dt, \quad \int_{t_o}^{t_f} J_L(t)\ dt,$$

where time is in milliseconds and the J (outward positive) are in µAcm^{-2}.

Question 2

If the integration time interval $[t_o\ ;\ t_f]$ contains only one action potential, as it should in the above examples, by executing the following command for any amplitude of the stimulating current,

```
>> [qna,qk,ql]=hhsplot(0,50);
```

what in general will be the relationship between $\int_{t_o}^{t_f} \sum_k J_k(t)\ dt$ and $\int_{t_o}^{t_f} J_{ei}(t)\ dt$ (allow for some numerical error) ?

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2. Refractoriness

For an interval of time, called the absolute refractory period, immediately after an action potential no second action potential can be elicited regardless of how intense the stimulus. Following the absolute refractory period is the relative refractory period during which a second action potential can be elicited for current strengths greater than the initial supra-threshold stimulus i.e. the relative refractory period is characterized by a progressively increasing threshold the closer one gets to the end of the absolute refractory period.

The space-clamped Hodgkin-Huxley equations also exhibit absolute and relative refractory periods. Further, as will be discussed in later lectures, the Hodgkin-Huxley equations are able to reveal the basis of such refractoriness.

To illustrate the features of the absolute and relative refractory periods we will stimulate our model axon with two current pulses separated by varying time intervals. Begin by setting the following parameters and executing `hhmplot`.

```
>> amp1 = 27.4;
>> width1 = 0.5;
>> delay2 = 25;
>> amp2 = 13.7;
>> width2 = 0.5;
>> hhsplot(0,40);
```

Both pulses should elicit action potentials. The threshold for a second pulse, $I_{1th} \approx 13.7$ µAcm^{-2} with the amplitude of the first pulse being set to twice this threshold value.

Question 3

By setting delay2 successively to 20, 18, 16, 14, 12, 10, 8 and 6 ms adjust amp2 to an accuracy of 0.1 µAcm^{-2} so as to just elicit an action potential. The amplitude so obtained, I_{2th}, will correspond to the current threshold amplitude for a second pulse as a function of the inter stimulus interval.

Question 4

By plotting the ratio I_{2th}/I_{1th} as a function of inter-pulse interval estimate the absolute and relative refractory periods.

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3. Repetitive activity

Long duration supra-threshold currents elicit multiple action potentials, a phenomenon called repetitive activity.

Question 5

By using either `hhsplot` or `hhmplot` estimate the number of action potentials per second by applying single 80 ms wide stimulus currents of 5, 10, 20, 30, 50, 70 and 100 µAcm^{-2}. Plot action potential frequency as a function of stimulating current amplitude. e.g
```
>> amp1 = 5;
>> width1 = 80;
>> delay2 = 0;
>> amp2 = 0;
>> width2 = 0;
>> hhmplot(0,100,0);
```

What changes do you notice in the amplitude of the action potentials as a function of stimulus intensity amplitude?

Question 6

Set the stimulating current amplitude to 200 µAcm^{-2}. What do you notice? This result is known as a depolarisation block. Can you think of an explanation of the results of Question 5 and Question 6 in terms of the voltage dependence of the *m, h* and *n* factors of the Hodgkin-Huxley equations?

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4. Temperature dependence

Measurements have established that ionic currents under voltage clamp have a faster time course at higher temperatures.

Question 7

By using a single current pulse of intensity 20 μAcm^{-2} and 0.5 ms in width observe the effects of the following temperatures on the duration and amplitude of the resulting action potential: 0, 5, 10, 15, 20, 24, 25, 26 and 30 ^{O}C. e.g

```
>> vclamp = 0;
>> amp1 = 20;
>> width1 = 0.5;
>> tempc = 0;
>> hhmplot(0,30,0);
>> hhsplot(0,30)
```

In general what features of the action potential are affected by increasing temperature?